

WHAT IS CLAIMED IS:

1. A method of culturing cells, comprising growing intestinal epithelial cells to form a confluent cell layer
5 in a culture media, wherein the intestinal epithelial cells express an enzyme activity selected from the group consisting of alkaline phosphatase, dipeptidyl peptidase IV, aminopeptidase and amino peptidase A, and wherein the culture media comprises:

10 a) an amount of a differentiating compound effective to maintain a reduced or physiologically meaningful transepithelial electrical resistance of the confluent cell layer; and

15 b) an amount of a kinase modulator effective in combination with the differentiating compound to support growth of microvilli longer than 1 micrometer on a portion of the epithelial cells.

20 2. The method of claim 1, wherein the differentiating compound is a fatty acid.

3. The method of claim 1, wherein the differentiating compound is a butyrate salt.

25 4. The method of claim 1, wherein the differentiating compound is sodium butyrate.

30 5. The method of any one of claims 1 through 4, wherein the effective amount of the differentiating compound is greater than 1mM.

6. The method of any one of claims 1 through 4, wherein the effective amount of the differentiating compound is from about 1 mM to about 10 mM.

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7. The method of any one of claims 1 through 4,

wherein the effective amount of the differentiating compound is about 5 mM.

8. The method of any one of claims 1 through 7,
5 wherein the kinase modulator is a kinase inhibitor.

9. The method of any one of claims 1 through 7,
wherein the kinase modulator has an activity selected from
the group consisting of activating JNK1, inhibiting GRK-5,
10 inhibiting PKC, inhibiting MAPKAP kinase-1beta and
inhibiting p70 S6 kinase.

10. The method of any one of claims 1 through 7,
wherein the kinase modulator is 2-{1-[3-(
15 amidinothio)propyl]-1H-indol-3-yl}-3-(1-methylindol-3-
yl)-maleimide methanesulfonate.

11. The method of any one of claims 1 through 10,
wherein the effective amount of the kinase modulator is
20 greater than 1 micromolar.

12. The method of any one of claims 1 through 10,
wherein the effective amount of the kinase modulator is
from about 1 micromolar to about 10 micromolar.
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13. The method of any one of claims 1 through 10,
wherein the effective amount of the kinase modulator is
about 5 micromolar.

30 14. The method of any one of claims 1 through 13,
wherein the confluent cell layer is grown on an
extracellular matrix base.

35 15. The method of claim 14, wherein the extracellular
matrix base comprises collagen.

16. The method of claim 14, wherein the extracellular matrix base comprises rat-tail collagen.

5 17. The method of any one of claims 1 through 16, wherein the intestinal epithelial cells are nontumorigenic.

18. The method of any one of claims 1 through 17, wherein the intestinal epithelial cells are human.

10 19. The method of any one of claims 1 through 17, wherein the intestinal epithelial cells are derived from a human duodenal biopsy.

15 20. The method of any one of claims 1 through 19, wherein the intestinal epithelial cells are derived from SCBN cells.

20 21. The method of any one of claims 1 through 20, wherein the transepithelial electrical resistance of the confluent cell layer is less than about 200 ohm.cm².

25 22. The method of any one of claims 1 through 20, wherein the transepithelial electrical resistance of the confluent cell layer is less than about 150 ohm.cm².

30 23. The method of any one of claims 1 through 20, wherein the transepithelial electrical resistance of the confluent cell layer is less than about 100 ohm.cm².

24. The method of any one of claims 1 through 23, wherein greater than 5% of the intestinal epithelial cells have microvilli longer than 1 micrometer.

35 25. The method of any one of claims 1 through 23, wherein greater than 10% of the intestinal epithelial cells

have microvilli longer than 1 micrometer.

26. The method of any one of claims 1 through 23,
wherein greater than 15% of the intestinal epithelial cells
5 have microvilli longer than 1 micrometer.

27. The method of any one of claims 1 through 23,
wherein greater than 20% of the intestinal epithelial cells
have microvilli longer than 1 micrometer.

10 28. The method of any one of claims 1 through 23,
wherein greater than 25% of the intestinal epithelial cells
have microvilli longer than 1 micrometer.

15 29. The method of any one of claims 1 through 23,
wherein greater than 30% of the intestinal epithelial cells
have microvilli longer than 1 micrometer.

20 30. The method of any one of claims 1 through 29,
wherein the culture media is essentially free of animal
serum.

25 31. The method of any one of claims 1 through 29,
wherein the culture media comprises D-glucose, L-glutamine,
sucrose, pyruvic acid, a pH buffer and one or more amino
acids.

30 32. The method of any one of claims 1 through 31,
wherein the culture is maintained at a temperature of about
36 degrees Celsius to 38 degrees Celsius.

33. An *in vitro* culture comprising a confluent layer
of intestinal epithelial cells, wherein:

35 a) the cells express an enzyme activity selected from
the group consisting of alkaline phosphatase, dipeptidyl

peptidase IV, aminopeptidase and amino peptidase A;
b) the confluent layer has a transepithelial
electrical resistance less than about 200 ohm.cm²; and
c) greater than 5% of the cells have microvilli
5 longer than 1 micrometer.

34. The culture of claim 33, wherein the culture is a
long-term culture having the enzyme activity,
transepithelial electrical resistance and microvilli of
10 claim 33 over a period of at least 48 hours starting about
72 hours after seeding the plates.

35. The culture of claim 33 or 34, wherein the
confluent layer is grown on an extracellular matrix base.
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36. The culture of claim 35, wherein the
extracellular matrix base comprises collagen.
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37. The culture of claim 35, wherein the
extracellular matrix base comprises rat-tail collagen.

38. The culture of any one of claims 33 through 37,
wherein the intestinal epithelial cells are nontumorigenic.
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39. The culture of any one of claims 33 through 38,
wherein the intestinal epithelial cells are human.
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40. The culture of any one of claims 33 through 39,
wherein the intestinal epithelial cells are derived from a
human duodenal biopsy.
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41. The culture of any one of claims 33 through 40,
wherein the intestinal epithelial cells are derived from
SCBN cells.
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42. The culture of any one of claims 33 through 41,
wherein greater than 5% of the intestinal epithelial cells

have microvilli longer than 1 micrometer.

43. The culture of any one of claims 33 through 41,
wherein greater than 10% of the intestinal epithelial cells
5 have microvilli longer than 1 micrometer.

44. The culture of any one of claims 33 through 41,
wherein greater than 15% of the intestinal epithelial cells
have microvilli longer than 1 micrometer.

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45. The culture of any one of claims 33 through 41,
wherein greater than 20% of the intestinal epithelial cells
have microvilli longer than 1 micrometer.

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46. The culture of any one of claims 33 through 41,
wherein greater than 25% of the intestinal epithelial cells
have microvilli longer than 1 micrometer.

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47. The culture of any one of claims 33 through 41,
wherein greater than 30% of the intestinal epithelial cells
have microvilli longer than 1 micrometer.

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48. A cell culture media capable of supporting an *in*
vitro culture comprising a confluent layer of intestinal
epithelial cells, wherein:

- a) the cells express an enzyme activity selected from the group consisting of alkaline phosphatase, dipeptidyl peptidase IV, aminopeptidase and amino peptidase A;
- b) the confluent layer has a transepithelial electrical resistance less than about 200 ohm.cm²; and
- c) greater than 5% of the cells have microvilli longer than 1 micrometer.

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49. A cell culture media comprising nutrients effective to support the viability of mammalian cells, and

further comprising sodium butyrate and 2-[1-[3-(amidinothio)propyl]-1H-indol-3-yl]-3-(1-methylindol-3-yl)-maleimide methanesulfonate.

5 50. The cell culture media of claim 49, wherein the effective amount of the kinase modulator is greater than 1 micromolar.

10 51. The cell culture media of claim 49, wherein the effective amount of the kinase modulator is from about 1 micromolar to about 10 micromolar.

15 52. The cell culture media of claim 49, wherein the effective amount of the kinase modulator is about 5 micromolar.

20 53. The cell culture media of any one of claims 49 through 52, wherein the effective amount of the differentiating compound is greater than 1mM.

54. The cell culture media of any one of claims 49 through 52, wherein the effective amount of the differentiating compound is from about 1mM to about 10mM.

25 55. The cell culture media of any one of claims 49 through 52, wherein the effective amount of the differentiating compound is about 5 mM.

30 56. The cell culture media of any one of claims 49 through 55, wherein the culture media is essentially free of animal serum.

35 57. The cell culture media of any one of claims 49 through 56, wherein the culture media comprises D-glucose, L-glutamine, sucrose, pyruvic acid a pH buffer and one or

more amino acids.

58. A method for determining if a culture media is capable of supporting an *in vitro* culture comprising a confluent layer of intestinal epithelial cells, wherein:

5 a) the cells express an enzyme activity selected from the group consisting of alkaline phosphatase, dipeptidyl peptidase IV, aminopeptidase and amino peptidase A;

10 b) the confluent layer has a transepithelial electrical resistance less than about 200 ohm.cm²; and c) greater than 5% of the cells have microvilli longer than 1 micrometer, comprising selecting a possible candidate media, contacting said media with SCBN and measuring the SCBN growth in said media to determine if a) the cells express an enzyme

15 activity selected from the group consisting of alkaline phosphatase, dipeptidyl peptidase IV, aminopeptidase and amino peptidase A; b) the confluent layer has a transepithelial electrical resistance less than about 200 ohm.cm²; and, c) greater than 5% of the cells have

20 microvilli longer than 1 micrometer.

59. The culture media determined by the method of claim 58 if not previously known.

25 60. A composition comprising an extract of the cell culture of any of claims 33-42.

61. The composition of claim 60, wherein the extract is an RNA extract.

30 62. A method for determining genes which control the expression of enzyme activity in a cell, wherein a) the enzyme activity is selected from the group consisting of alkaline phosphatase, dipeptidyl peptidase IV, aminopeptidase and amino peptidaseA ;

35 b) the cell has a transepithelial electrical resistance less than about 200 ohm.cm²; and,

c) when the said cells are grown in a culture comprising the following steps, greater than 5% of the cells have microvilli longer than 1 micrometer:

- 5 i) growing the SimBioDAS[®] cells in a culture; and
ii) characterizing the genes expressed in said culture to determine which genes control the above expression,

63. The genes determined by the method of claim 62 if they are not previously known.

10 64. A composition comprising the genes determined by the method of claim 62 in an effective concentration capable of transforming appropriate cells to control the expression of enzyme activity in a cell, wherein a) the enzyme activity is selected from the group consisting of alkaline phosphatase, dipeptidyl peptidase IV, 15 aminopeptidase and amino peptidase A; b) the cell has a transepithelial electrical resistance less than about 200 ohm.cm²; and c) when the said cells are grown in a culture, greater than 5% of the cells have microvilli longer than 1 micrometer.

20 65. A method for preparing the cell culture of claim 33-42 comprising introducing the genes capable of controlling the expression of an enzyme activity in a cell, wherein a) the enzyme activity is selected from the group 25 consisting of alkaline phosphatase, dipeptidyl peptidase IV, aminopeptidase and amino peptidase A; b) the cell has a transepithelial electrical resistance less than about 200 ohm.cm²; and c) when the said cells are grown in a culture, greater than 5% of the cells have microvilli longer than 1 30 micrometer.

66. The method of claim 65, wherein the genes are recited in Table 2.

35 67. The cell lines derived from the cell culture

prepared by the method of claim 64.

68. A method of processing an aqueous composition comprising a plurality of compounds to obtain a physiologically modified composition, the method comprising: applying the aqueous composition to a first side of an *in vitro* confluent intestinal epithelial cell layer; and, extracting the modified composition from a second side of the confluent intestinal epithelial cell layer; wherein:

a) cells of the confluent intestinal epithelial cell layer express an enzyme activity selected from the group consisting of alkaline phosphatase, dipeptidyl peptidase IV, aminopeptidase and amino peptidase A;

b) the confluent intestinal epithelial cell layer has a transepithelial electrical resistance less than about 200 ohm.cm²; and,

c) a portion of the cells of the confluent intestinal epithelial cell layer have microvilli longer than 1 micrometer.

69. The method of claim 68, wherein cells of the confluent intestinal epithelial cell layer are nontumorigenic.

70. The method of claim 68 or 69, wherein cells of the confluent intestinal epithelial cell layer are human.

71. The method of any one of claims 68 through 70, wherein cells of the confluent intestinal epithelial cell layer are derived from a human duodenal biopsy.

72. The method of any one of claims 68 through 71, wherein cells of the confluent intestinal epithelial cell

layer are derived from SCBN cells.

5 73. The method of any one of claims 68 through 72, wherein the transepithelial electrical resistance of the confluent cell layer is less than about 200 ohm.cm².

10 74. The method of any one of claims 68 through 73, wherein greater than 5% of the intestinal epithelial cells have microvilli longer than 1 micrometer.

15 75. The method of any one of claims 68 through 74, wherein the aqueous composition comprising a plurality of compounds is a plant extract.

20 76. The method of any one of claims 68 through 75, wherein the confluent intestinal epithelial cell layer is grown in a culture media comprising:

25 a) an amount of a differentiating compound effective to maintain the transepithelial electrical resistance of the confluent cell layer; and,

30 b) an amount of a kinase modulator effective in combination with the differentiating compound to support growth of microvilli longer than 1 micrometer on the apical portion of the cells.

35 77. A physiologically modified composition produced by the method of any one of claims 68 through 76.

78. A method of treating intestinal epithelial cells comprising administering to the cells a differentiating compound in combination with an amount of 2-{1-[3-(amidinothio)propyl]-1H-indol-3-yl}-3-(1-methylindol-3-yl)-maleimide methanesulfonate effective to support growth of microvilli longer than 1 micrometer on a portion of the epithelial cells.

79. An epithelial cell culture substantially as hereinbefore described and with reference to the examples and drawings.

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80. A ginkgo extract processed according to the method of any one of claims 68 through 76.

81. A ginkgo extract substantially as hereinbefore 10 described and with reference to the examples and drawings.

82. A profile generated by the physiologically modified composition of claim 77.

15 83. The profile of claim 82 indicating that the composition is therapeutically useful.

84. A method to determine the batch variation of a natural product comprising steps of:

20 a) generating a profile of claim 83; and
b) comparing the profile of each batch of the product to determine whether said batch would be therapeutically useful.

25 85. A method to identify needed absorbable active ingredients by further purification of the ingredients identified in the profile of claim 82.

30 86. The absorbable active ingredient identified by the method of claim 85.

87. A composition comprising the absorbable active ingredient of claim 86.

35 88. A pharmaceutical composition comprising the

absorbable active ingredient of claim 86 and a pharmaceutically acceptable carrier.

5 89. A method for treating a subject comprising administering an effective amount of the pharmaceutical composition of claim 88 to the subject.

10 90. The gene expression profile derived from the microarray study using the SimBioDAS[®] cell.

15 91. The gene expression profile of claim 90, used to identify the cell signaling pathway involved in SimBioDAS[®] cell differentiation.

15 92. The signaling pathway identified by claim 91 if it is not previously known.

20 93. A method for manipulation of the cell signaling pathways of SimBioDAS[®] cells through genetic manipulation or application of kinase inhibitors/activators in the tissue culture medium to achieve fuller cell differentiation by the information of the gene expression profile of claim 91 or the signaling pathway of claim 92.

25 94. The manipulated cell of claim 91.

95. The extract of the manipulated cell of claim 91.

30 96. A chemical compound screening method of processing an aqueous composition comprising a solution containing a chemical drug candidate compound to obtain a physiologically modified composition, the method comprising: applying the aqueous composition to a first side of an *in vitro* confluent intestinal epithelial cell layer; and, extracting the modified composition from a

second side of the confluent intestinal epithelial cell layer; wherein:

5 a) cells of the confluent intestinal epithelial cell layer express an enzyme activity selected from the group consisting of alkaline phosphatase, dipeptidyl peptidase IV, aminopeptidase and amino peptidase A;

10 b) the confluent intestinal epithelial cell layer has a transepithelial electrical resistance less than about 200 ohm.cm²; and,

15 c) a portion of the cells of the confluent intestinal epithelial cell layer have microvilli longer than 1 micrometer.

15 97. The method of claim 94, wherein cells of the confluent intestinal epithelial cell layer are nontumorigenic.

20 98. The method of claim 96 or 97, wherein cells of the confluent intestinal epithelial cell layer are human.

25 99. The method of any one of claims 96 through 98, wherein cells of the confluent intestinal epithelial cell layer are derived from a human duodenal biopsy.

100. The method of any one of claims 96 through 99, wherein cells of the confluent intestinal epithelial cell layer are derived from SCBN cells.

30 101. The method of any one of claims 96 through 100, wherein the transepithelial electrical resistance of the confluent cell layer is less than about 200 ohm.cm².

35 102. The method of any one of claims 96 through 101, wherein greater than 5% of the intestinal epithelial cells

have microvilli longer than 1 micrometer.

5 103. The method of any one of claims 96 through 102, wherein the aqueous composition comprising a solution of a compound is a chemical compound being screened as a drug candidate.

10 104. The method of any one of claims 96 through 103, wherein the confluent intestinal epithelial cell layer is grown in a culture media comprising:

- a) an amount of a differentiating compound effective to maintain the transepithelial electrical resistance of the confluent cell layer; and,
- b) an amount of a kinase modulator effective in combination with the differentiating compound to support growth of microvilli longer than 1 micrometer on the apical portion of the cells.

15 20 105. A physiologically modified composition produced by the method of any one of claims 96 through 104.